Tunneling nanotubes (TNTs) are tube-shaped cell membrane structures between cells.[2] The characterization of TNTs' functionality by traditional biological approaches often suffers from the complexities and a lack of controllability in the connection. We developed synthetic TNTs by connecting cells with the LNTs as a model system to investigate this new cell-cell communication tool. Cells (REF52 and PC12) were rinsed with physiological buffer solution to remove associated proteins, and seeded on surface-patterned LNTs. Dye-tagged phospholipids incorporated in the LNTs diffused into the cell plasma membrane, suggesting the fusion between the LNTs and the cell membrane. Physical parameters (*e.g.* temperature, osmotic pressure) were optimized to promote the fusion efficiency. TNTs were found to mediate the calcium wave propagation in different cells types. It implies that the inter-cellular calcium migration does not require the direct cell-cell contact with formation of gap junctions but occurs remotely through TNTs over much longer distance. We study this TNT-mediated calcium propagation by fabricating several artificial TNT-cell circuits of different geometries.

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2818-Plat

Sorting of tN-Ras by Membrane Curvature in Lipid Vesicles and Tubes

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Ras proteins are small GTPases that are post-translationally modified by the attachment of lipid moieties (1). This modification is essential for the correct trafficking and sorting of Ras proteins through the vesicular pathway from the Golgi to the plasma membrane (2).
Traditionally the sorting of Ras is primarily discussed in the context of membrane domains in flat membranes, neglecting the influence of membrane shape. Recently we have demonstrated, utilizing our single liposome curvature (SLiC) assay that the minimal anchoring motif of N-Ras (tN-Ras) up-concentrates in areas of high membrane curvature (not published), suggesting that curvature might act as a cue for the spatial localization of Ras proteins.
In the SLiC assay, curvature-sensing molecules are added from aqueous solution to vesicles of different curvatures, but the vesicles are not in diffusive contact (3, 4). In vivo Ras proteins are anchored to membranes and laterally sorts between curved and planar membranes, which are in diffusive contact. To study the curvature-sensing ability of tN-Ras in a setup mimicking the in vivo scenario we developed a membrane tube based assay in which the tubes are in diffusive contact with a lipid bilayer.
Membrane tubes are formed by heating a confined lipid bilayer (5). The tubes eventually adsorb to the flat membrane, which enable imaging by confocal fluorescence microscopy. After addition of tN-Ras we observed a preferential sorting into curved tubes rather than the flat bilayer. This observation further implies a pivoting role of membrane shape as a regulator of Ras-protein localization.

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2819-Plat

Modulation of Membrane Rigidity by Human and Yeast Homologs of the Vesicle Trafficking Protein Sar1

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Understanding how proteins manipulate the shape and form of membranes is crucial to intracellular cargo trafficking, yet the mechanical activities of trafficking proteins remain poorly understood. using an optical-trap based assay involving dynamic membrane deformations and fluorescence recovery after photobleaching (FRAP) to measure protein mobility on in vitro endoplasmic reticulum mimic membranes, we examined the behavior of the two human paralogs of Sar1, a key component of the COPII family of vesicle coat proteins. Like their yeast (S. cerevisiae) counterpart, the human Sar1 proteins can lower the mechanical rigidity of the membranes to which they bind. Unlike the yeast Sar1, the rigidity is not a monotonically decreasing function of concentration. At high concentrations, we find increased bending rigidity and decreased protein mobility. These features imply a model in which protein clustering influences membrane mechanical properties. Additionally we are investigating other membrane-associated proteins known to cluster in order to further our understanding of the model and the effects these proteins have on rigidity and mobility.

2820-Plat

Sound Propagation in Lipid Membranes

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It has recently been shown that in-plane sound waves can propagate over long distances in lipid monolayers [1]. Earlier it has been proposed that the propagation of nerve signals can be described by sound phenomena called solitons [2]. The implications of sound propagation in lipid membranes for signaling in biology are far reaching and insight into this is essential for further investigation. Particularly interesting are propagation properties in the vicinity of the biologically relevant lipid melting transition, where mechanical and thermodynamical properties of the system change drastically. We have theoretically addressed the properties of sound propagation in lipid membranes throughout the lipid melting transition. We explored dispersion and attenuation for low frequency sound propagation, a regime previously unexplored. We find that dispersion and attenuation is closely related to the relaxation and the state of lipid membranes [3]. Interestingly, the vast significant changes of dispersion and attenuation occur on timescales similar to ion channel open times and the temporal length of the nerve pulse.

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2821-Plat

Are Phospholipids Stabilized by Short, Strong H-Bonds?

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Short-strong H-bonds are defined as H-bonds between anions such as the carboxyls of maleate held proximal by a proton and maleate's cis-doublebond. They are stabilized by the combined resonance of the anions they connect.
Bilayers in living membranes are typically stabilized by P-lipids or glycolipids (G-lipids). G-lipids, examined by Xray & NMR, show that in addition to the extensive van der Waals bonding between chains, the headgroups have multiple H-bonding between the sugars. P-lipids can be zwitterionic, PC & PE. However most P-lipids (PG, PS, PI, etc.) have anionic repulsive headgroup interactions. Yet bilayers made of P-lipids have physical properties, Tm's, etc., similar to those of the G-lipids. In chloroplasts and many bacterial membranes all of the P-lipids are either uncharged (G-lipids) or anionic! Nonetheless the Xray (& NMR) data both show that all the P-lipid headgroup conformations are identical: 3 glycerol carbons perpendicular to the membrane plane with the phosphate on top and the primary chain ester directly below them. The polar R-group is attached to the apical phosphate. This high density of surface anions must result (Guoy-Chapman) in a high density of protons at the uniformly erect phosphates at the membrane surface. Together with the high proton density this suggests short-strong H-bonds between the phosphates such as those formed in stable oleic acid bilayers (Haines, TH, PNAS 80,160 (1983)) and in cardiolipin conformed in bilayers. (Haines, BBA-Biomem-branes 1788, 1997-2002 (2002)) In the data shown in the PNAS article, the number of protons oleate needs to form bilayers varies from 20 to 80% of the total anions (carboxyls). I propose that the erect phosphates trap fleeting protons between the anions, stabilizing a phosphate ionic sheet for bilayer formation such as occurs in oleic acid bilayers.

Platform: Actin, Microtubules, and their Binding Proteins

2822-Plat

Molecular Origins of Cofilin-Linked Changes in Actin Filament Mechanics

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The actin regulatory protein, cofilin, plays a central role in actin assembly dynamics by severing filaments and increasing the concentration of ends from which subunits add and dissociate. Cofilin binding modifies the average structure and mechanical properties of actin filaments, thereby promoting